DTI of tuber and perituberal tissue can predict epileptogenicity in tuberous sclerosis complex

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ABSTRACT

Objective: To evaluate whether diffusion tensor imaging (DTI) can predict epileptogenic tubers by measuring apparent diffusion coefficient (ADC), fractional anisotropy, axial diffusivity, and radial diffusivity in both tubers and perituberal tissue in pediatric patients with tuberous sclerosis complex (TSC) undergoing epilepsy surgery.

Methods: We retrospectively selected 23 consecutive patients (aged 0.4–19.6 years, mean age of 5.2; 13 female, 10 male) who underwent presurgical DTI and subsequent surgical resection between 2004 and 2013 from the University of California–Los Angeles TSC Clinic. We evaluated presurgical examinations including video-EEG, brain MRI, ^{18}F -fluorodeoxyglucose-PET, magnetic source imaging, and intraoperative electrocorticography for determining epileptogenic tubers. A total of 545 tubers, 33 epileptogenic and 512 nonepileptogenic, were identified. Two observers generated the regions of interest (ROIs) of tubers ($\text{ROI}^{\text{tuber}}$), the 4-mm-thick ring-shaped ROIs surrounding the tubers ($\text{ROI}^{\text{perituber}}$), and the combined ROIs ($\text{ROI}^{\text{tuber+perituber}}$) in consensus and calculated maximum, minimum, mean, and median values of each DTI measure in each ROI for all tubers.

Results: The Mann–Whitney U test demonstrated that the epileptogenic group showed higher maximum ADC and radial diffusivity values in all ROIs, and that maximum ADC in $\text{ROI}^{\text{tuber+perituber}}$ showed the strongest difference ($p = 0.001$). Receiver operating characteristic analysis demonstrated that maximum ADC measurements in $\text{ROI}^{\text{tuber+perituber}}$ (area under curve = 0.68 ± 0.05 , $p < 0.001$) had 81% sensitivity and 44% specificity for correctly identifying epileptogenic tubers with a cutoff value of $1.32 \mu\text{m}^2/\text{ms}$.

Conclusions: DTI analysis of tubers and perituberal tissue may help to identify epileptogenic tubers in presurgical patients with TSC more easily and effectively than current invasive methods. *Neurology*® 2015;85:2011–2015

GLOSSARY

AD = axial diffusivity; **ADC** = apparent diffusion coefficient; **DTI** = diffusion tensor imaging; **FA** = fractional anisotropy; **FDG-PET** = ^{18}F -fluorodeoxyglucose-PET; **RD** = radial diffusivity; **ROC** = receiver operating characteristic; **ROI** = region of interest; **TSC** = tuberous sclerosis complex; **UCLA** = University of California–Los Angeles.

The presurgical identification of epileptogenic tubers in patients with tuberous sclerosis complex (TSC) remains challenging.¹ Many patients with TSC either require invasive intracranial recording to pinpoint the epileptogenic zone or are denied surgery altogether.

Recent neurophysiologic studies have revealed that not only the tuber themselves but also the adjacent perituberal tissue can impair brain function and is therefore considered part of the ictal onset zone.^{2,3} We hypothesized that microstructural changes in perituberal tissue may be measurable using diffusion tensor imaging (DTI), an MRI technique sensitive to subvoxel microstructural orientation and density. To test this hypothesis, we measured DTI characteristics, including apparent diffusion coefficient (ADC), fractional anisotropy (FA), axial diffusivity (AD), and radial diffusivity (RD), in both tubers and perituberal tissue in a group of pediatric patients with TSC, and then correlated these findings with epileptogenicity.

Supplemental data
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METHODS Standard protocol approvals, registrations, and patient consents. The institutional review board at the University of California–Los Angeles (UCLA) approved the use of human subjects and waived the need for written informed consent and signed patient consent-to-disclose form because all testing was deemed clinically relevant to patient care.

Ethical approval. The current retrospective study was approved by the institutional review board at UCLA.

Patients. We retrospectively selected 23 consecutive pediatric patients with TSC (aged 0.4–19.6 years mean age of 5.2; 13 female, 10 male) who underwent presurgical DTI and subsequent surgical resection for treatment of epilepsy between 2004 and 2013 from the UCLA TSC Clinic. Two patients underwent surgery twice because of localizable recurrent seizures, arising from adjacent to resection cavity. Patients' clinical data and seizure characteristics are summarized, respectively, in tables e-1 and e-2 on the *Neurology*® Web site at Neurology.org. None of the patients had a history of treatment with mammalian target of rapamycin inhibitors at the time of MRI or surgery. Patients without surgical resection due to nonlateralizing or multiple independent epileptogenic zones were excluded (n = 11).

Selecting epileptogenic tubers. All patients underwent a standardized presurgical evaluation including clinical and neurologic examinations, interictal and ictal scalp video-EEG recordings, brain MRI, and ¹⁸F-fluorodeoxyglucose–PET (FDG-PET). FDG-PET/MRI coregistration⁴ was performed

for all patients, and 18 of the patients received magnetic source imaging⁵ for interictal dipoles. Epileptologists, neurosurgeons, neuroradiologists, and neuropsychologists made the decisions regarding surgical candidacy by group consensus during weekly case conferences.

We reviewed these preoperative examinations and intraoperative electrocorticography to confirm epileptogenic areas. Also, we verified the specific tubers responsible for seizure activity subsequently through surgical resection and a clinically meaningful reduction in seizure activity. Since magnetic susceptibility-induced artifacts on MRI cause inaccurate DTI measurements, we excluded heavily calcified tubers from the analysis. A total of 545 tubers, consisting of 33 (6%) epileptogenic tubers and 512 (94%) nonepileptogenic tubers were identified in this study.

Neuroimaging acquisition and analysis. We used the DTI data and other conventional magnetic resonance sequences on 1.5T Siemens Signa HDx or Genesis scanner and 3T Siemens Trio scanner (Siemens AG, Erlangen, Germany) and processed the analysis using the freely available postprocessing software AFNI (<http://afni.nimh.nih.gov/afni>) (figure 1). Detailed magnetic resonance protocols and measures are shown in appendix e-1. Two researchers manually contoured all tubers on the ADC maps slice by slice in consensus (region of interest [ROI]^{tuber}). Because of the low resolution of the ADC maps, we referred T2-weighted or fluid-attenuated inversion recovery images overlaying on ADC maps. ROI^{tuber} was circumferentially inflated by 4 mm, such that at least 2 voxel rows were included

Figure 1 Creating the ROIs of tuber, perituberal tissue, and combined tuber and perituberal tissue

A 2-year-old boy with tuberous sclerosis complex. Axial T2-weighted image (A), axial T1-weighted image (B), and axial apparent diffusion coefficient map (C) showed multiple bihemispheric cortical tubers. ROI of left frontal tuber (ROI^{tuber}) was generated by manually contouring the tuber by 2 observers in consensus (D). ROI^{tuber} was automatically inflated by 4 mm to create the ROI of tuber plus perituberal tissue (ROI^{tuber+perituber}) (E). Note that the regions over CSF of ROI^{tuber+perituber} were trimmed off. ROI^{tuber} was subtracted from ROI^{tuber+perituber} to generate the ROI of perituberal tissue (ROI^{perituber}) (F). The in-plane voxel size for DTI data in our study ranged from 0.8 × 0.8 to 1.9 × 1.9 mm. In order to include at least 2 voxel rows in the perituberal ROIs, a width of 4 mm was used. ROI = region of interest.

around the tubers, to create ROI^{tuber+perituber}. Finally, original ROI^{tuber} was then subtracted from ROI^{tuber+perituber} to create the ROI of perituberal tissue (ROI^{perituber}). Flow voids, CSF, bone, and air were manually eliminated from all ROIs. Maximum, minimum, mean, and median values of each DTI measure in each ROI were then calculated for all tubers.

Statistical analysis. We entered DTI measurements into a database and analyzed the data using SPSS Statistics, version 22 (IBM Corp., Armonk, NY). A priori, results were considered significant at $p < 0.05$.

RESULTS The results of the group analysis are summarized in table 1. All patients showed improvement in seizure activity postoperatively, all of which corresponded to those defined as epileptogenic on preoperative images (table e-1).

Epileptogenic tubers showed higher maximum ADC and RD values in all ROIs (Mann–Whitney U test; p values ≤ 0.03). Maximum ADC and RD were different between each ROI (Bonferroni; $p < 0.001$), and there was no interaction effect between ROI and epileptogenicity (2-way analysis of variance; $p = 0.12$) (figure e-1). Maximum AD values of epileptogenic tubers were higher in ROI^{perituber} (Mann–Whitney

U test; $p = 0.03$) and ROI^{tuber+perituber} (Mann–Whitney U test; $p = 0.04$). FA values showed no significant differences.

The results suggested that the maximum ADCs and RDs in ROI^{tuber+perituber} showed the strongest differences between epileptogenic and nonepileptogenic tubers ($p = 0.001$ and 0.01 , respectively). Receiver operating characteristic (ROC) analysis demonstrated that these DTI characteristics differentiate epileptogenic tubers with high sensitivity (figure 2). In particular, maximum ADC measurements in ROI^{tuber+perituber} (ROC, area under curve = 0.68 ± 0.05 , $p < 0.001$) demonstrated 81% sensitivity and 44% specificity for correctly identifying epileptogenic tubers with a cutoff value of $1.32 \mu\text{m}^2/\text{ms}$ (figure 2A), and maximum RD measurements in ROI^{tuber+perituber} (ROC, area under curve = 0.63 ± 0.05 , $p = 0.01$) demonstrated 84% sensitivity and 37% specificity with a cutoff value of $1.1 \mu\text{m}^2/\text{ms}$ (figure 2B).

DISCUSSION The current study suggests that DTI measurements, especially maximum ADC and maximum RD, in both tuber and perituberal tissues can

Table 1 Comparison of ROI measurements

Measurement	ROI ^{tuber}			ROI ^{perituber}			ROI ^{tuber+perituber}		
	Epileptogenic	Nonepileptogenic	p Value	Epileptogenic	Nonepileptogenic	p Value	Epileptogenic	Nonepileptogenic	p Value
ADC									
Min.	0.88 ± 0.12	0.93 ± 0.11	0.03 ^a	0.77 ± 0.73	0.78 ± 0.89	0.77	0.77 ± 0.08	0.78 ± 0.09	0.69
Max.	1.60 ± 0.43	1.36 ± 0.32	<0.01 ^a	1.39 ± 0.25	1.28 ± 0.24	0.02 ^a	1.66 ± 0.39	1.41 ± 0.31	0.001 ^a
Mean	1.18 ± 0.21	1.12 ± 0.16	0.09	0.98 ± 0.08	0.97 ± 0.10	0.51	1.05 ± 0.13	1.02 ± 0.14	0.13
Median	1.16 ± 0.20	1.10 ± 0.16	0.10	0.97 ± 0.08	0.96 ± 0.10	0.60	1.02 ± 0.11	0.99 ± 0.11	0.25
FA									
Min.	0.09 ± 0.03	0.11 ± 0.05	0.21	0.09 ± 0.03	0.09 ± 0.03	0.61	0.07 ± 0.03	0.08 ± 0.03	0.28
Max.	0.40 ± 0.14	0.36 ± 0.12	0.09	0.49 ± 0.15	0.47 ± 0.12	0.53	0.50 ± 0.16	0.47 ± 0.12	0.39
Mean	1.95 ± 0.38	0.21 ± 0.06	0.46	0.27 ± 0.13	0.24 ± 0.09	0.31	0.22 ± 0.04	0.22 ± 0.06	0.75
Median	1.87 ± 0.38	0.20 ± 0.62	0.44	0.22 ± 0.04	0.21 ± 0.06	0.59	0.21 ± 0.04	0.21 ± 0.06	0.96
AD									
Min.	1.00 ± 0.33	1.00 ± 0.26	0.52	0.84 ± 0.20	0.85 ± 0.20	0.71	0.83 ± 0.20	0.85 ± 0.21	0.50
Max.	1.72 ± 0.56	1.57 ± 0.50	0.17	1.79 ± 0.48	1.63 ± 0.52	0.02 ^a	1.90 ± 0.58	1.68 ± 0.56	0.03 ^a
Mean	1.32 ± 0.34	1.25 ± 0.31	0.15	1.15 ± 0.23	1.13 ± 0.25	0.32	1.22 ± 0.26	1.16 ± 0.27	0.11
Median	1.31 ± 0.34	1.23 ± 0.30	0.15	1.13 ± 0.22	1.11 ± 0.25	0.32	1.18 ± 0.26	1.14 ± 0.26	0.17
RD									
Min.	0.69 ± 0.23	0.73 ± 0.22	0.21	0.57 ± 0.19	0.58 ± 0.18	0.42	0.56 ± 0.20	0.58 ± 0.18	0.41
Max.	1.45 ± 0.56	1.20 ± 0.42	0.02 ^a	1.28 ± 0.37	1.14 ± 0.35	0.03 ^a	1.50 ± 0.53	1.26 ± 0.42	0.01 ^a
Mean	1.02 ± 0.35	0.95 ± 0.28	0.33	0.83 ± 0.21	0.81 ± 0.21	0.50	0.90 ± 0.26	0.85 ± 0.23	0.26
Median	1.00 ± 0.33	0.94 ± 0.27	0.37	0.82 ± 0.21	0.80 ± 0.21	0.61	0.87 ± 0.23	0.83 ± 0.22	0.41

Abbreviations: AD = axial diffusivity; ADC = apparent diffusion coefficient; FA = fractional anisotropy; Max. = maximum; Min. = minimum; RD = radial diffusivity.

Data are presented as mean $\pm$ SD ($\times 10^{-3} \text{ mm}^2/\text{s}$).

^a $p < 0.05$.

Figure 2 ROC curves for identifying epileptogenicity based on maximum ADC and maximum RD within each ROI

ROC analysis results for (A) maximum ADC and (B) maximum RD within the tuber, perituberal tissue, and combined tuberal and perituberal tissues. Results demonstrate a high sensitivity for identifying epileptogenic tubers if maximum ADC is higher than $1.32 \mu\text{m}^2/\text{ms}$ in tubers (72% sensitivity/53% specificity), perituberal tissue (59% sensitivity/62% specificity), or combined tuberal and perituberal tissue (81% sensitivity/44% specificity). Similarly, a maximum RD higher than $1.1 \mu\text{m}^2/\text{ms}$ in tubers (75% sensitivity/42% specificity), perituberal tissues (68% sensitivity/50% specificity), or combined tuber and perituberal tissues (84% sensitivity/37% specificity) showed a high sensitivity for correctly identifying epileptogenic tubers. ADC = apparent diffusion coefficient; RD = radial diffusivity; ROC = receiver operating characteristic; ROI = region of interest.

enhance the identification of epileptogenic tubers. Histopathologically, tubers show the presence of dysplastic neurons and giant cells, increased axonal connectivity, and hypomyelination.³ Perituberal cortex also demonstrates similar histologic features.³ Our results are therefore consistent with these histopathologic changes since dysplastic neurons and astrogliosis may result in increased ADC. Cystic degeneration of tubers also causes increased ADC, which is consistent with the notion that tubers with cystic degeneration correlate with epileptogenicity.⁶ It is possible that the functional isolation of the tuberal regions by cystic white matter may promote epileptogenicity. In future studies, it would be beneficial to directly analyze the relationship of imaging/DTI findings to electrocorticographic findings and histopathologic features.

In addition, hypomyelination is reported to increase RD, which is consistent with our results.⁷ AD changes are caused by axonal and functional changes,⁷ suggesting that our observed increases in AD within epileptogenic perituberal tissue may result from increased axonal connectivity and growth.³ The coexistence of both AD and RD changes have resulted in no substantial anisotropy changes, as indicated by our findings of no FA difference between epileptogenic and nonepileptogenic tubers. This result conflicts with previous studies reporting significantly lower FA values in epileptogenic tubers or in normal-appearing white matter adjacent to epileptogenic tubers compared with nonepileptogenic areas.^{8,9} Those studies, however, determined epileptogenicity using α -[¹¹C]methyl-L-tryptophan

([¹¹C]AMT)-PET⁸ or magnetic source imaging,⁹ each of which is probably insufficient for determining epileptogenicity alone. They also used small focal ROIs to measure DTI indices in normal-appearing white matter⁹ while we created ring-shaped ROIs covering all of the perituberal tissue simultaneously, which enabled us to reflect all of the FA changes within perituberal tissue. Consequently, we can say that the current results reflect all FA changes in perituberal tissue, and that FA change may vary according to where in the perituberal area the FA values were measured. In addition, it is well known that the major white matter tracts, including the internal capsule and corpus callosum, show significant FA decrease in patients with TSC compared with those in normal subjects.¹⁰ Concordant with our results, epileptogenic activity may cause more severe FA decrease in the hemisphere with epileptogenic tubers.

Limitations of this study include its retrospective design, and exclusion of patients who have not undergone surgical resection. Despite these limitations, this study demonstrates that DTI analysis of tuber and perituberal tissue may help identify epileptogenic tubers in patients with TSC noninvasively. Because this is a preliminary study, this method has not yet been adopted clinically. Further evaluation with a larger sample may help identify epileptogenic tubers noninvasively.

AUTHOR CONTRIBUTIONS

Drs. Yogi, Hirata, Salamon, and Mathern designed the study and drafted the manuscript. Drs. Yogi, Hirata, Karavaeva, Ellingson, and Salamon and Mr. Harris processed the image analyses. Drs. Yogi, Hirata, Wu, and Salamon and Ms. Yudovin collected and evaluated clinical data. Drs. Yogi,

Ellingson, and Salamon processed the statistical analysis and reviewed the results. All authors reviewed the study design and edited the manuscript.

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DISCLOSURE

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